

# ART 34 AMDT

## WHAT IS CLAIMED IS:

1. A DNA demethylase enzyme and/or homologue thereof, which comprises an amino acid sequence set forth in SEQ ID NOS:2, 4, 6 and 8 and homologue thereof, and wherein said DNA demethylase enzyme has about 40 KDa and is overexpressed in cancer cells.
2. A cDNA encoding a human demethylase of claim 1, which comprises a sequence set forth in SEQ ID NOS:1 and 3.
3. A cDNA homologous to the cDNA of claim 2, wherein said cDNA encoding mouse demethylase set forth in SEQ ID NOS:5 and 7.
4. *a* The use of the expression of demethylase cDNA of claims 2 ~~or 3~~ to alter DNA methylation patterns of DNA in vitro in cells or in vivo in humans, animals and in plants.
5. The use of claim 4, wherein said demethylase cDNA expression is under the direction of mammalian promoters.
6. The use of claim 5, wherein said promoter is CMV.
7. The use of claim 4, wherein said demethylase cDNA expression is under plant specific promoters to alter methylation in plants and to allow for altering states of development of plants and expression of foreign genes in plants.
8. The use of claim 4, wherein said demethylase cDNA expression is in the antisense orientation to inhibit demethylase in cancer cells for therapeutic processes.

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9. The use of claim 9, wherein expression of demethylase cDNA in mammalian cells is to alter their differentiation state and to generate stem cells for therapeutics, cells for animal cloning and to improve expression of foreign genes.

a 10. The use of the expression of demethylase cDNA of claims 2 ~~or 3~~ in bacterial or insect cells for production of large amounts of demethylase.

a 11. The use of the expression of demethylase cDNA of claims 2 ~~or 3~~ for the production of protein in vertebrate, insect or bacterial cells.

12. The use of claim 11 for producing antibodies against demethylase.

13. The use of the sequence of demethylase cDNA of claim 2 as a template to design antisense oligonucleotides and ribozymes.

14. The use of the predicted peptide sequence of demethylase cDNA of claim 2 to produce polyclonal or monoclonal antibodies against demethylase.

a 15. The use of expression of cDNA of claim 2 ~~or 3~~ in two hybrid systems in yeast to identify proteins interacting with demethylase for diagnostic and therapeutic purposes.

a 16. The use of expression of cDNA of claim 2 ~~or 3~~ in bacterial, vertebrate or insect cells to produce large amounts of demethylase for high throughput screening of

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demethylase inhibitors for therapeutics and biotechnology and for obtaining the x-ray crystal structure.

17. A volatile assay for high throughput screening of demethylase inhibitors as therapeutics and anticancer agents which comprises the steps of:

- a) using transcribed and translated demethylase cDNA of claim 2 or 3 *in vitro* to convert methylcytosine present in methylated DNA samples to cytosine present in DNA and volatilize methyl group;
- b) determining the absence or minute amount of volatilized methyl group as an indication of an active demethylase inhibitor.

18. A volatile assay for the diagnostics of cancer in a patient sample which comprises the steps of:

- a) determining demethylase activity in patient samples by determining conversion of methylcytosine present in methylated DNA to cytosine present in DNA and volatilization of the methyl group released as methanol;
- b) determining the presence or minute amount of volatilized methyl group as an indication of cancer in said patient sample.

19. Use of an antagonist or inhibitor of DNA demethylase of claim 1 ~~or 2~~ for the manufacture of a medicament for cancer treatment, for restoring an aberrant methylation pattern in a patient DNA, or for changing a methylation pattern in a patient DNA.

20. Use according to claim 19, wherein said antagonist is a double stranded oligonucleotide that inhibits demethylase at a  $K_i$  of 50nM.

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21. Use according to claim 20, wherein said oligonucleotide is  $\begin{matrix} [C^mGC^mGC^mGC^mG] \\ [G^mCG^mCG^mCG^mC]_n \end{matrix}$

22. Use according to claim 19, wherein the inhibitor comprises an anti-DNA demethylase antibody or an antisense oligonucleotide of DNA demethylase or a small molecule.

*Claim*  
a 23. Use according to ~~one of claims 19 or 22~~, wherein the change of the methylation pattern activates a silent gene.

24. Use according to claim 23, wherein the activation of a silent gene permits the correction of genetic defect.

25. Use according to claim 24, wherein said genetic defect is  $\beta$ -thalassemia or sickle cell anemia.

26. Use of the demethylase of claim 1, for removing methyl groups on DNA *in vitro*.

a 27. Use of the demethylase of claim 1 ~~or its cDNA of claim 2~~, for changing the state of differentiation of a cell to allow gene therapy, stem cell selection or cell cloning.

a 28. Use of the demethylase of claim 1 ~~or its cDNA, of claim 2~~ for inhibiting methylation in cancer cells using vector mediated gene therapy.

29. An assay for the diagnostic of cancer in a patient, which comprises determining the level of expression of DNA demethylase of claim 1 in a sample

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from said patient, wherein overexpression of said DNA demethylase is indicative of cancer cells.

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